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The identification of osmoprotectant compounds from *Jatropha curcas* Linn. plant for natural drought stress tolerance

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Abstract

One of the plant species that its seed can produce oil for biodiesel material is *Jatropha curcas* Linn. The using of *J. curcas* plant for supporting the conversion of fossil fuel to biofuel and the utilization for dry land in Indonesia is very prospective. However, it is necessary to create *J. curcas* varieties that are tolerant to drought stress. The objective of this study was to identify the content of osmoprotectant compounds in *J. curcas* as a natural tolerance to drought stress. The plants sampled were six weeks old by isolation of the sap from plant stems and then followed by Gas Chromatography–Mass Spectrometry (GC–MS) analysis. This study was found to be suspected of 15 osmoprotectant compounds in the sap of *J.curcas* namely glycine, serine, proline, valine, threonine, leucine, asparagine, salicylic acid, glutamine, methionine, phenylalanine, arginine, tyrosine, raffinose, and stachyose. The three main of osmoprotectant compounds were suspected as stachyose (8.40%), raffinose (6.78%) and leucine (2.64%), while the low level of osmoprotectant included salicylic acid (0.16%), and followed by lycine, serine and methionine with the similar content (0.24%). It is therefore, in the future *J. curcas* can potentially be utilized for dry land reforestation and biofuel production.

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Keywords: Biodiesel; Drought stress; *Jatropha curcas Linn*; Osmoprotectant; Stachyose

1. Introduction

Indonesia still imports oil fuel that increases yearly, along with the increasing consumption of fuel oil and the decline in oil production. The dependence on fossil energy, especially petroleum in Indonesia is very high from the total national energy consumption. Therefore, the developing of renewable energy from biofuels is important.

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One of the plants that potential to produce biodiesel fuel in the tropical region is *J. curcas* Linn. The potency of *J. curcas* will support the conversion of fossil oil to biofuel. Hence, planting of *J. curcas* for biodiesel and utilization of dry land in Indonesia is important. First and foremost, selecting plants varieties that tolerant to drought stress is still needed. *J. curcas* can live more than 25 years and the seeds contain oil up to 35% and are easily converted to biodiesel [1]. *J. curcas* seed production will be stable after the plants are more than 5 years old depends on the quality of the seeds, agro-climate, soil fertility, and plant maintenance. *J. curcas* seed oil can be obtained by pressing the seeds and following by extraction process if necessary.

Groundwater is one of the most critical environmental components and required in a large quantities for plant growth and development. Water loss in plant tissue will reduce cell turgor, increase the concentration of various macromolecular compounds and influence on cell membranes and water activity in the plant cells. In the previous research showed that the highest production of *J. curcas* can achieved during optimum irrigation. Many factors also contribute to improve crop production in sub-optimal land, i.e. improving plant genetic capacity, developing production management systems, developing infrastructures, and increasing the capacity of technical and farmers institution as an actors of crop production [2].

Lack of water can interfere physiological and morphological activities of the plants, thus inhibiting growth and influencing plant production. Drought is an abiotic factor limits growth and productivity that can result in a reduction of photosynthesis rate so that growth and productivity levels are low [3]. Assembling the drought-tolerant plants is essential for the development of marginal land [4]. The purpose of this study to identify the content of osmoprotectant compounds in *J. curcas* plants for natural tolerance to drought stress.

2. Materials and methods

This study was using *J. curcas* genotypes of JCUMM5 crossbred plant that was selected for 3 years at dry land condition [5,6]. For the plants sampling were selected 100 genotypes. Selected plants were propagated in a greenhouse using cuttings planting material measuring around to 30 cm length and 2–2.5 cm in diameter.

The study was carried out in a greenhouse facility and the Laboratory of Vegetable Oil Technology, University of Muhammadiyah Malang. The content analysis of secondary metabolites was carried out when the *J. curcas* plant was six weeks old by isolation of the sap from plant stems. Furthermore, the analysis of osmoprotectant compounds was modified using modified Gas Chromatography–Mass Spectrometry (GC–MS) Shimadzu QP 5000 [7]. The content analysis of osmoprotectant was applied two replications or duplo analysis.

3. Results and discussions

Plant reactions to drought stress are different depending on plant species, growth rate, plant tolerance, intensity and duration of stress. Osmoprotectant compounds are compounds that will enable when the plant has experienced an osmotic stress, by maintaining the water content in the tissue. Due to inability of plant tissue to handle this situation, thus affecting on cell shrinkage.

The response of each plant genotype in producing osmoprotectant compounds varies in the drought stress condition. Based on the Gas Chromatographic–Mass Spectrometry (GC–MS) test using sap from the stem of *J. curcas* plant, 15 compounds that have been obtained were suspected as osmoprotectants, namely glycine, serine, proline, valine, threonine, leucine, asparagine, salicylic acid, glutamine, methionine, phenylalanine, arginine, tyrosine, raffinose and stachyose (Table 1).

From fifteen osmoprotectant compounds that were obtained, the three high-level of osmoprotectant contents were stachyose (8.40%), followed by raffinose (6.78%) and leucine (2.64%). The low-level of contents were salicylic acid (0.16%), followed by glycine, serine, and methionine with similar content as much as 0.24% (Table 1).

Furthermore, Table 2 shows the names of compounds, formulas, molecular weight, molecular structure and various similarity indexes for osmoprotectant compounds in *J. curcas* plant.

Drought stress conditions generally will increase the proline content in plants. Other compounds that correlated with drought stress including salicylic acid, glycine, tyrosine, leucine, and valine. In corn plants, drought stress causes an increase in the number of free amino acids and the accumulation of valine, asparagine, serine, and threonine [8]. Previous study has also found the expressions of high valine in Arabidopsis plants [9]. The physicochemical analysis shows that drought-stressed leaves shows accumulate in free amino acids [10]. Glutamine and arginine are found increased in conditions of water stress and salt stress, so these compounds are considered

Table 1. The composition and osmoprotectants content of *J. curcas* plant (minimum, maximum and average in percent from entire compound)..

No.	Osmoprotectant compounds	Osmoprotectant content (%)		Average of osmoprotectant content (%)
		Minimum	Maximum	
1.	Glycine	0.23893	0.25812	0.24
2.	Serine	0.23820	0.26228	0.24
3.	Proline	0.92609	1.13725	1.69
4.	Valine	1.38863	1.52833	1.44
5.	Threonine	1.38953	1.56830	1.45
6.	Leusine	2.53961	2.80271	2.64
7.	Asparagine	0.46926	0.51641	0.48
8.	Salicylic Acid	0.15628	0.15628	0.16
9.	Glutamine	0.92689	1.09653	0.99
10.	Methionine	0.23610	0.26115	0.24
11	Phenil alanine	1.84914	2.04574	1.92
12	Arginine	2.07603	2.28738	2.16
13	Tyrosine	0.92343	1.10676	0.99
14	Raffinose	6.53891	6.98278	6.78
15	Stachyose	8.10169	8.68643	8.40

as osmoprotectants [11]. Table 2 also shows that the osmoprotectant compounds have a molecular weight varying from 75.07 to 666.58. But, the entire compound has a similarity index around 92%.

The plant can adapt to environmental stresses such as drought. The response of plants to adapt to drought stress varies between genotypes. Some plants that have drought stress will accumulate a certain amount of proline. The effect of drought stress on proline accumulation has been studied in beans [12].

The response of *J. curcas* plants to adapt to drought stress varies between genotypes. Some study report that under drought stress conditions can increase proline compound [13] (Verslues and Sharma, 2010). Production and accumulation of proline in plants are not always occurred in drought stress conditions [14]. Some studies have found other compounds that can be suspected of dealing with drought stress, such as salicylic acid, glycine, tyrosine, leucine, and valine. Besides that in some plants, there are other compounds such as raffinose that produced from precursors [15].

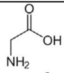
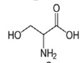
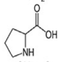
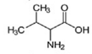
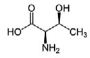
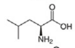
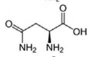
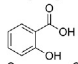
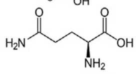
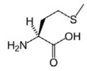
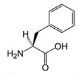
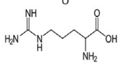
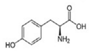
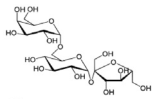
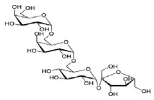
Drought stress is a term to state that a plant has experienced a condition of water shortages due to limited water availability from the growing media [16]. Drought stress in plants can be caused by a lack of water supply in the root area and excessive water demand by leaves due to the rate of evapotranspiration that exceeds the rate of absorption of water even though the condition of groundwater is sufficiently available. Water stress in plants can be caused by two factors, included lack of water in the root area and higher evapotranspiration rate compared to the rate of absorption by plant roots. Therefore, the water needs of the leaves are higher than any other parts. Environmental factors and plant physiological factors will influence water absorption by plants. Environmental factors that influence includes soil water content, air humidity, and soil temperature. Plant physiological factors include root efficiency, a difference in diffusion pressure of groundwater to roots, and protoplasmic condition of plants.

The accumulation of proline amino acid will occur due to a plant in response to environmental stresses such as temperature, drought and other extreme conditions of growth factors. The high level of proline makes it possible to maintain low water potential. Decreasing water potential allows plants to take water from the environment [17]. The adaptability of plants for other drought stress is sugar compound. This compound functions for plant growth and development. In drought stress, plants generally accumulate dissolved of high sugars compounds to reduce the growth rate [18]. Sugar can protect the membrane integrity during dehydration by preventing membrane fusion, a transition phase, and membrane separation phase.

4. Conclusions

This results of this study showed that fifteen compounds that suspected osmoprotectant in *J. curcas* plant from sap, i.e. glycine, serine, proline, valine, threonine, leucine, asparagine, salicylic acid, glutamine, methionine, phenylalanine, arginine, tyrosine, raffinose, and stachyose. The three main compounds of osmoprotectants were indicated as stachyose (8.40%), raffinose (6.78%) and leucine (2.64%), while the low contents were salicylic acid

Table 2. The name of compounds, formulas, molecular weight, molecular structure and various similarity indexes for osmoprotectant compounds in *J. curcas* plant.

No.	Name of compound	Formula	Molecular weight	Molecular structure	Similarity Index (%)
1.	Glycine	$C_2H_5NO_2$	75.07		92
2.	Serine	$C_3H_7NO_3$	105.09		92
3.	Proline	$C_5H_9NO_2$	115.13		92
4.	Valine	$C_5H_{11}NO_2$	117.15		92
5.	Threonine	$C_4H_9NO_3$	119.12		92
6.	Leucine	$C_6H_{13}NO_2$	131.18		92
7.	Asparagine	$C_4H_8N_2O_3$	132.12		92
8.	Salicylic acid	$C_7H_6O_3$	138.12		92
9.	Glutamine	$C_5H_{10}N_2O_3$	146.15		92
10.	Methionine	$C_5H_{11}NO_2S$	149.21		92
11.	Phenilalanine	$C_9H_{11}NO_2$	165.19		92
12.	Arginine	$C_4H_{14}N_4O_2$	174.20		92
13.	Tyrosine	$C_9H_{11}NO_3$	181.19		92
14.	Raffinose	$C_{18}H_{32}O_{16}$	504.44		92
15.	Stachyose	$C_{24}H_{42}O_2$	666.58		92

(0.16%), followed by glycine, serine, and methionine content (0.24%). Hence, this finding could be as consideration for planting *J. curcas* in dry land or marginal land conditions as well as an effort to produce biofuels plant.

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References

[1] Pompelli MF, Barata-Luís RM, Vitorino HS, Gonçalves ER, Rolim EV, Santos MG, Almeida-Cortez JS, Ferreira VM, Lemos EEP, Endres L. Photosynthesis, photoprotection and antioxidant activity or purging nut under drought deficit and recovery. Biomass Bioenergy 2010;34:1207–15.

[2] Sobir. Optimalisasi Lahan Sub Optimal bagi Penguatan Ketahanan Pangan Indonesia [Sub Optimal Land Optimization for Strengthening Indonesian Food Security] [in Bahasa Indonesia] Proceeding of Sub Optimal Land National Seminar, Sriwijaya University Palembang Indonesia; 2013. p. 23-8. http://docplayer.info/53155936-Prosiding-seminar-nasional-lahan-suboptimal-tema-intensifikasi-pengelolaan-lahan-suboptimal-dalam-rangka-mendukung-kemandirian-pangan-nasional.htm. [Accessed 22 March 2017].

[3] Johari-Pireivatlou M. Effect of soil water stress on yield and proline content of four wheat lines. African J Biotec 2010;9(1):036–40. http://dx.doi.org/10.5897/AJB09.521, https://academicjournals.org/journal/AJB/article-full-text-pdf/122F8E026296.

[4] Behera SK, Srivastava P, Triphati R, Singh JP, Singh N. Evalution of plant performance of Jatropha curcas Linn under different agro-practice for optimizing biomass-A case study. Biomass Bioenergy 2010;34:30–41. http://dx.doi.org/10.1016/j.biombioe.2009.09.008, https://www.semanticscholar.org/paper/Evaluation-of-plant-performance-of-Jatropha-curcas-Behera-Srivastava/54b37e7fd1453f0357c75bbbbd715bbb94add747.

[5] Maftuchah A, Zainudin, Sudarmo H. Production of physic nut hybrid progenies and their parental in various dry land. Agric Sci 2013;4(1):48–56. http://dx.doi.org/10.4236/as.2013.41008.

[6] Maftuchah, Reswari HA, Ishartati E, Zainudin A, Sudarmo H. Heretability and correlation of vegetative and generative character on genotypes of Jatropha (Jatropha curcas Linn.). Energy Procedia 2015;65:186–93. http://dx.doi.org/10.1016/j.Egypro.2015.01.058.

[7] Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of evolvulus alsinoides L. J Food Sci Technol 2015;52(2):1212–7. http://dx.doi.org/10.1007/s13197-013-1105-9, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4325072/pdf/13197_2013_Article_1105pdf.

[8] Ranieri A, Bernardi R, Lanese P, Soldatini PF. Changes in free amino acid content and protein pattern of maize seedlings under water stress. Environ Exp Bot 1989;29:351–7, https://www.sciencedirect.com/science/article/abs/pii/0098847289900099.

[9] Rizhsky L, Liangand HJ, Shuman J, Shulaev V, Davletova S, Mittler R. When defense pathways collide. The response of arabidopsis to a combination of drought and heat stress. Plant Physiol 2004;134:1683–96, Published 2004 http://dx.doi.org/10.1104/pp.103.033431, http://www.plantphysiol.org/content/plantphysiol/134/4/1683.full.pdf.

[10] Showler AT, Moran PJ. Effects of drought stressed cotton, Gossypium hirsutum L. on beet armyworm, spodoptera exigua (hubner), oviposition and larval feeding preferences and growth. J Chem Ecology 2003;29(9):1997–2011, https://naldc.nal.usda.gov/download/41712/PDF.

[11] Sun CX, Li MQ, Gao XX, Liu LN, Wu XF, Zhou JH. Metabolic response of maize plants to multi-factorial abiotic stresses. Plant Biol 2015. http://dx.doi.org/10.1111/plb.12305, https://onlinelibrary.wiley.com/doi/full/101111/plb.12305#accessDenialLayout.

[12] Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian J Crop Sci 2010;4(8):580–5, https://www.researchgate.net/publication/48186785_Effect_of_drought_stress_on_yield_proline_and_chlorophyll_contents_in_three_chickpea_cultivars.

[13] Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids 2008;35(4):753–9. http://dx.doi.org/10.1007/s00726-008-0061-6, Epub. 2008 Apr 1 https://www.ncbi.nlm.nih.gov/pubmed/18379856.

[14] Guevara DR, Champigny MJ, Tattersall A, Dedrick J, Wong CE, Li Y, Labbe A, Ping CL, Wang YX, Nuin P, Golding G, McCarry BE, Summers PS, Moffatt BA, Weretilnyk EA. Transcriptomic and metabolomic analysis of Yukon Thellungiellaplants grown in cabinets and their natural habitat show phenotypic plasticity. BMC Plant Biol 2012;12:175–92.

[15] Taiz L, Zeiger E. Plant Physiology. U.S.: Sinauer Associates. 2002. p. 33–67. http://exa.unne.edu.ar/biologia/fisiologia.vegetal/PlantPhysiologyTaiz2002.pdf.

[16] Sharma S, Verslues PE. Mechanisms independent of ABA or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. Plant, Cell Environ 2010;33:1838–51. http://dx.doi.org/10.1111/j.1365-3040.2010.02188.x.

[17] Kumar SG, Matta Reddy A, Sudhakar C. NaCl affects on proline metabolism in two high yield genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Sci 2003;165:1245–51, https://www.academia.edu/17263839/NaCl_effects_on_proline_metabolism_in_twohigh_yielding_genotypes_of_mulberry_Morus_alba_L._with_contrasting_salt_tolerance.

[18] Riahi M, Ehsanpour AA. Responses of transgenic tobacco (*Nicotiana plambaginifolia*) over expressing P5CS gene under in vitro salt stress. Progress Biol Sci 2012;2(2). https://pbiosci.ut.ac.ir/article_2708_cd5713eaae0e282571a434b3a1957c5a.pdf.